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The Flower Structure and Anther Dehiscence of *Wolffia arrhiza* (Lemnaceae)

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ABSTRACT: Light and electron microscopic studies were undertaken on the flower structure and anther dehiscence of *Wolffia arrhiza* L. The anthesis is protogynous. The pistil and the stamen were located in dorsal flower cavity and the pistil protrude first. The inner structure of the pistil and the stamen described with special notes on the anther dehiscence line and the septal cells. The pistil is comprised of a concave stigma, a style and a unilocular ovary with one orthotropous ovule. The bitegumic ovule is covered with a secretary substance within the ovary. The stamen has a filament and bilobed monothecous anther. The tannin body in the stomial cells as well as stigma and style may act as a deterrent. Meanwhile, the uneven thickening of the septal walls and the massive central vacuole of the septal cells probably aid the dehiscence of the anther sac.

KEY WORDS: Flower structure, Lemnaceae, Stomia, *Wolffia*.

INTRODUCTION

The watermeals (*Wolffia* Hook. ex Schleid.) are the smallest aquatic angiosperms in the world. Characteristics such as small size, a special budding structure, short life cycle and easy culture, render them favorable for plant morphogenetic and physiological studies. The flowering process has been studied in some species of this genus (Gupta, 1935; Maheshwari, 1954; Rimon and Galun, 1968; Anderson *et al.*, 1973; Pan and Chen, 1979; Bernard *et al.*, 1990; Landolt, 1992 and White and Wise, 1998). Gupta (1935) briefly described the development of pollen grain and the embryo sac of *Wolffia arrhiza* L. The embryology and morphogenesis of *W. microspica* (Griff.) Kurz have been studied with light microscopy (Maheshwari, 1954; Rimon and Galun, 1968). Bernard *et al.* (1990) represented the morphology of flower of *W. australiana* (Benth.) Hartog and Plus by using light and scanning electron microscopy, while Landolt (1992) specified experimentally induced flowers of the same species. In the present investigation, we describe some additional information about the flower of *W. arrhiza* with both light and electron microscopy.

MATERIALS AND METHODS

A mixed culture of *W. arrhiza*, *Lemma minor* L. and *Spirodela polyrhiza* (L.) Sculeid was collected from a farm field, cultivated with *Trapa* sp., near Guan-tien, Tainan, in June 1993. The sample was maintained in pond water in glass tanks without aeration. Tap water was added periodically to replace water lost through evaporation. The culture appeared

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healthy throughout the fall and winter of 1993 and 95. Samples for microscopy were selected from the culture tanks. The taxon was identified by using Yang's key (1978).

For scanning electron microscopy, samples of *W. arrhiza* were fixed as the procedure of Pan and Chen (1979). In brief, whole plants were immersed in a fixative consisting of a 17:2:1 (v:v:v) mixture of 20% ethanol, 10% glacial acetic acid, and 50% formalin for 12 hours at room temperature. Dehydration was through a graded alcohol-acetone series and critical-point drying was in a Hitachi HCP-2 CPD unit using liquid CO_2. Specimens were lightly coated with gold and then viewed in a Hitachi S 2500 scanning electron microscope at 25 kV.

For light and transmission electron microscopy, samples were fixed for 2-4 hours in glutaraldehyde (2.5% in 0.1 M phosphate buffer, pH 7.0), rinsed twice in buffer, and then postfixed for 2-4 hours in osmium (1% in the same buffer). All steps were at room temperature. Samples were dehydrated through a graded acetone series and embedded in Spurr's epoxy (Spurr, 1969). Thick sections (1.0 μm) were stained with warm toluidine blue for light microscopy. Sections were viewed and photographed with light microscopy. Thin sections (70-90 nm) were stained with ethanolic urnyal acetate and calcined lead citrate (Haniachi *et al.*, 1986). Sections were viewed and photographed on a Zeiss EM10C transmission electron microscope at 60 kV.

RESULTS

Flower structure and flowering

The *W. arrhiza* contains a pistil and a stamen situated in it's dorsal flower cavity (Fig. 1A). The round flower cavity has small epidermal cells along its margin (Fig. 2B), and it is partial separated by some cells near its base (Fig. 2C). The pistil, which grows on the anterior side, is comprised of a concave stigma, a style and a unilocular ovary with one orthotropous ovule (Figs. 2A & 2D). The bitegumic ovule is covered with a secretary substance within the ovary (Fig. 2 D). The stamen has a filament and bilobed monothecous anther (Figs. 1E & 2A). The anther has a dehisecence line (Fig. 1E).

Usually the pistil matures earlier than the stamen. At first, the pistil protrudes from the cavity when flowering (Figs. 1B-C). Some cells of the stigma and style accumulate tannin gradually (Fig. 2A), then the stigma and style wilt as vestige near the cavity opening, and the stamen gradually grows up and obliquely protrudes from the cavity by the elongation of the filament (Figs. 1D-E; Fig. 2E). Gradually, the anther opens from the dehiscense line, two lobes separate then curve outwards and downwards to expos the entire mass of pollen grains for dissemination (Fig. 1 F). The pollen grain has an exine with sharply conical echinae and one obscure germinating pore (Figs. 1G-H).

Anther walls and anther dehiscence

Three layers of the anther wall: the epidermis, the endothecium and tapetum are recognized before anthesis (Fig. 2A). The epidermal cells are greatly stretched and flattened. The endothecial layer acquires prominent fibrous thickenings. The protoplasts of the tapetal cells wander inside the locule to form a true periplasmodium (Fig. 2A). The periplasmodium is gradually consumed as the pollen grains mature. (Fig. 3A). The cells of stomium accumulate tannin and begin to degenerate (Fig. 3B). The septal cells have massive central vacuole and uneven thickening cell walls (Fig. 3C). The endothecial layer, the stomium and the septal cells may facilitate the opening of the anther at anthesis.

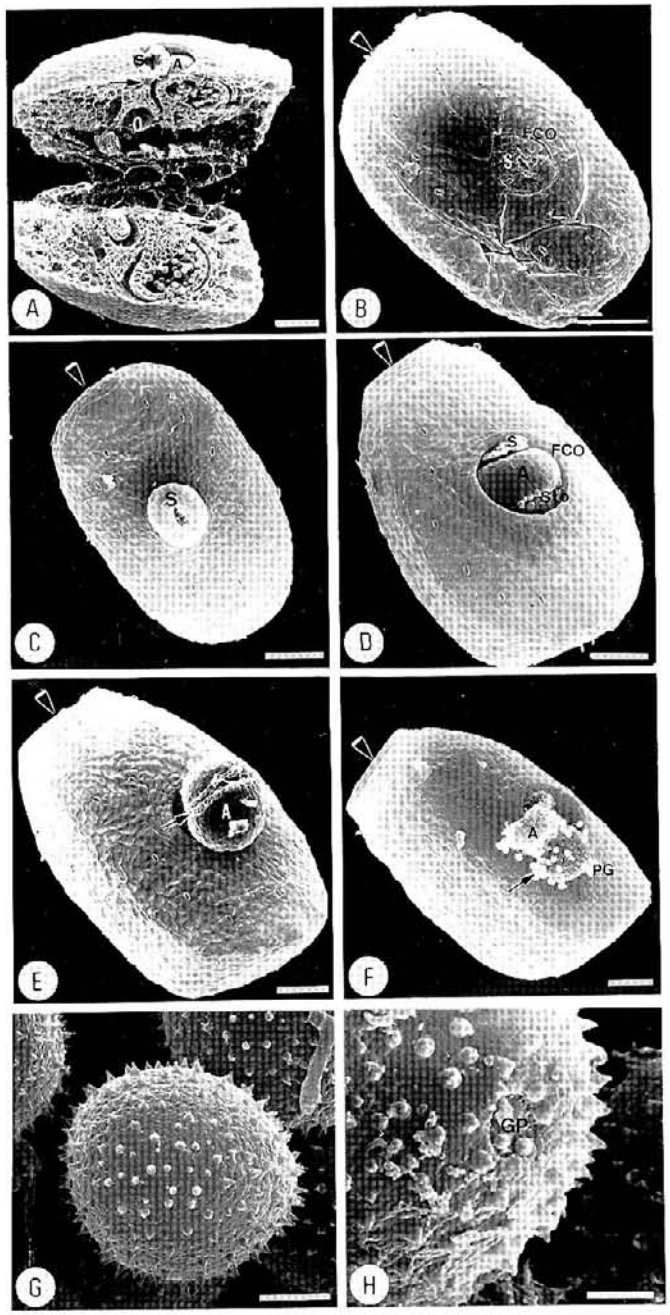

Fig. 1. SEM of flowers and pollen of *Wolffia arrhiza*. (A-F, bar=100 μm; G, bar=5 μm; H, bar=2 μm). All mother fronds with the budding cavity (arrowhead) face to left or upper left corner of photographs A-F. A. Longtudinal section showing pistil and stamen (A) in flower cavity. Note pistil where stigma (S), style (arrow) and ovary (O) are visible. Daughter fronds (asterisk) can be seen in the budding cavity. B. Flowering frond with a pistil before protruding from the flower cavity opening (FCO). Note a ring of slit between stigma (S) and FCO is visible. C. Flowering frond with an open expanded stigma (S) protruding from the flower cavity opening (covering by the stigma). D. Flowering frond with an wilting stigma (S) and a fresh anther (A) with a dehiscence line (Sto) before protruding from the flower cavity opening (FCO). E. Flowering frond with a bilocular anther (A). Note the dehiscence line (arrow), which aid in dehiscence. F. Flowering frond with dehisced anthers (A). Note the opening anther lobes with numerous pollen grains (arrow). G. Spinulose pollen grains. H. Pollen grain with one germinating pore (GP).

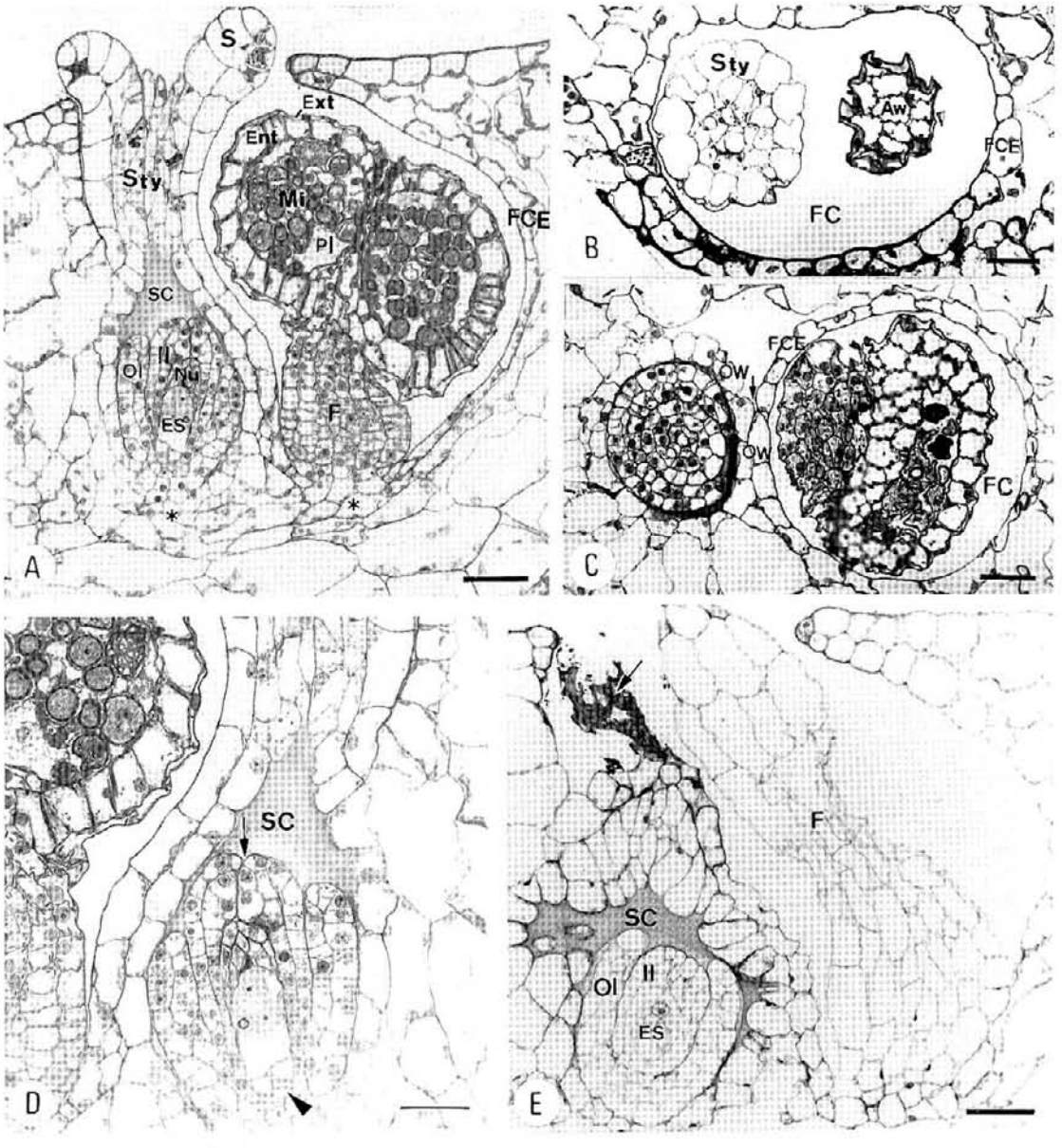

Fig. 2. Light micrograph of thick section of *Wolffia arrhiza*. (A, E, bar=50 μm; B-C, bar=60 μm; D, bar=5 μm). A. Longitudinal section, showing pistil and stamen stands upright on a cushion of parenchymatous tissue (asterisks) at the base of the flower cavity. Note the flask-shaped ovary with a style (Sty) which connect the stigma (S). Inside the ovary, an ovule with outer integument (OI) and inner integument (II) enclosed the nucellus (Nu) and embryo sac (Es) are visible. Stamen of this stage, showing short filament (F) and massive anther with flattened epidermis (Ext), endothecium (Ent), plasmodium tapetal cells (Pl) and microspores (Mi). B. Paradermal section through style (Sty) and part of anther wall (Aw). Note the solid style with 3-4 layers of cell, and the round flower cavity (FC) with small epidermal cells (FCE) along its margin. C. Paradermal section through flower cavity (FC) at the level of ovule and base of anther sac. Note the stamen and ovary are partially separated near the base by portion of ovary wall (OW) and flower cavity epidermis (FCE). D. Logitudinal section showing the orthotropus ovule with eight nucleated embryo sac. Note 3 antipodal cells at chalaza end (arrowhead), 2 polar nuclei, and the egg apparatus at the micropylar end (arrow). At this stage, the ovary cavity still full with secretary substance (SC). E. Longitudinal section showing the pistil and filament (F). Note the upper part of pistil (arrow) is wilt, however the ovary cavity still full with secretary substance (SC), and the ovule develop well with outer integument (OI), inner integument (II) and embryo sac (ES).

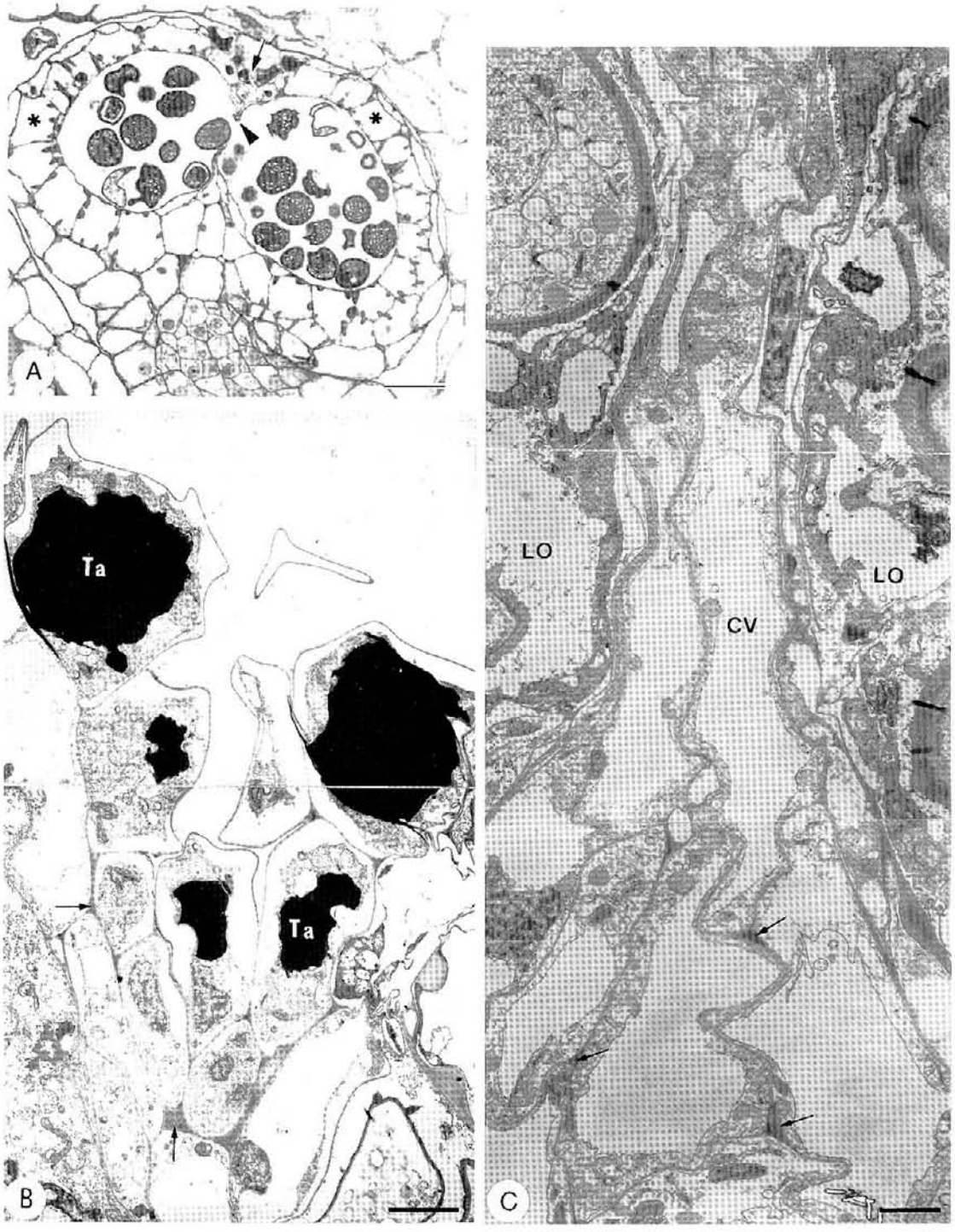

Fig. 3. Light micrograph of stamen, and TEM micrographs of stomium and septum. (A, B, bar=5 μm; C, bar=2 μm). A. Light micrograph of longitudinal section of a stamen, showing the stomium (arrow), endothecium (asterisk) and septum (arrowhead) of th anther. Note the stomial cells contain tannin (arrow), the endothecial cells have fibrous thickening, and the septal cells are degenerated. B. TEM of stomial cells and parts of septal cells (bar=5 μm). Note the stomial cells contain tannin (Ta), and the septal cell walls are uneven thickening (arrow). C. TEM of septal cells between two loculi (Lo), showing the large central vacuole (CV) and uneven thickening of the cell wall (arrow).

DISCUSSION

Like many other species, there is a single pistil and a single stamen situated in the *W. arrhiza* flower cavity, whatever the different interpretation of the flower (Hillman, 1961; Landolt, 1986). Moreover, the structures of both female and male parts are similar in most species of the *Wolffia*. However, several flower types were reported by Landolt (1992) in experimentally induced flowers of *W. australiana*.

Hillman (1961) and Landolt (1986) noted that there is confusion as to whether *Wolffia* and *Lemna* should be interpreted as producing a perfect flower or two separate female and male flowers. Most authors prefer the former interpretation (Hillman, 1961; Maheshwari and Chauhan, 1963; Bernard *et al.*, 1990; Landolt, 1992). However, Hartog and Van der Plas (1970) stated that the inflorescence of the subfamily *Wolffioideae* contains 1 female and 1 male flower, without a spathe. Hillman (1961) pointed out the problem mentioned above and argued that there was a lack of conclusive evidence. Although Rimon and Galun (1968) clearly show the presence of two floral primordia in the early stage of flower development in *W. microscopica*, we found some partial separation between the pistil and the stamen near the basal part inside the common flowering cavity. This feature hints the question of one or two separate flowers requires further study.

The inner structures of the *Wolffia* flowers are also without conspicuous differences. The anther wall comprises the thin epidermial cells, the fibrous thickening endothecial cells and the amoeboid tapetal cells. The major feature, the absence of the middle layer, is a comparatively rare feature in angiosperms in *W. arrhiza* (Gupta, 1935) and *W. microscopica* (Maheshwari, 1954). However, this must be confirmed by studying the early anther wall formation since Johri *et al.* (1992) presented contray opinions. The *W. arrhiza* pistil is comprised of a concave stigma, a style and a unilocular ovary with one orthotropous, bitegumic ovule. Although these carpel features are very similar to a previous report (Gupta, 1935; Maheshwari, 1954), we found that the style is solid instead of hollow as specified by Gupta (1935). Moreover, the secretary substance full within the ovary cavity, a character also found in *Pistia*, and the accumulation of tannin in some cells of both the stigma and style in the pistil are also noteworthy. The tannin cells may act as a deterrent (Fahn, 1982). The tannin cells wilt immediately after the pistil anthesis, leaving more room for the stamen to protrude from the very narrow opening of the flower cavity. In addition, the asymmetrical growth of both the anther sacs and the filament are also an adjustment for obliquely ascending the larger anther from the smaller opening.

A dehiscence line found on the stamen, which was noted by numerous authors, aided in opening the anther (Gupta, 1935; Maheshwari, 1954; Bernard, *et al.*, 1990; Lindolt, 1992). However, their structure is not represented in any clear detail. The stomial cells on the dehiscence line accumulated extensive amounts of tannin in the present study. This tannin could act as a deterrent that prevents insects'feeding on the anthers (Fahn, 1982; Mauseth, 1988). We also observed the uneven thickening walls and the massive central vacuole in the septal cells of the *W. arrhiza* anther. These features probably aid the septal cells in their separation and in dehiscence of the anther sac when they accomapany the endothecium.

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無根萍（浮萍科）之花部構造與花藥開裂

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摘　　要

應用光學及電子顯微鏡檢觀察無根萍之花的內部構造與花藥開裂。無根萍於葉狀體背部花腔內先後分別長出雌蕊和雄蕊，通常為雌先熟。除就雌蕊和雄蕊內部構造詳加描述外，特別探討花藥開裂線及藥隔之細胞特性。開裂線上的口邊細胞、柱頭及花柱細胞內富含單寧，可能具保護防禦功能。藥隔之細胞其細胞壁加厚不均勻且具大型液胞，此等特性與花藥內壁纖維質增厚可能共同促成花藥之開裂。

關鍵詞：花部構造、浮萍科、口邊細胞、無根萍。

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